ANNEXURES I

RAMA MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

MANDHANA KANPUR

CONSENT FORM

I .....son/daughter/wife of.....exercising my free power of choice, hereby give my consent for information related to me to be included in the study "Clinical Impact of Nosocomial Infection due to *Acinetobacter baumannii* in Intensive Care Units with Antimicrobial Resistance and Molecular Characterisation.

I understand that my name and identity will be concealed.
Once signed, I cannot revoke my consent.

(To be explain in detail in native language)

सहमति फार्म

मैं .....पुत्र /पुत्री /पत्नी/..... स्वयं इस अध्ययन में भाग लेना रहा/रहीं हूं I मैं स्वयं से संबंधी सूचना इस अध्ययन कार्य में प्रकाशित करने की सहमति देता/देती हूं I मैं समझता/ समझती हूं कि मेरा पहचान गोपनीय रखी जाएगी।

एक बार हस्ताक्षर के पश्चात मैं सहमति रद्द नहीं कर सकता/ सकती हूं I

सहभागी

नाम:.....

हस्ताक्षर/अगुठे का निशान:.....

दिनाक:.....

ANNEXURES II

PATIENT PROFORMA

Clinical and Microbiological profile of patient

Clinical Profile:

Name of Patient: …… …. Sex / Age:….....**.....**

Laboratory No/Sample No.: ……..**.....**Address: ….....

Microbiological Profile:

DAY 1

Time of sample collection: ……..…..Specimen: .....……

Macroscopic Observation: Color: ……..... Appearance: …… pH…… Others: ……..

Direct Microscopic Observation:

Direct Gram Staining / Wet mount (For urine):**.....**

Culture of specimen on: a. MacConkey Agar b. Blood Agar c. CLED Agar (For Urine)

Incubation temperature and period: …….

DAY 2: Reading of Culture Plates

Colony Characteristics on MacConkey Agar /Blood Agar

Media used	Shape	Size	Color	Texture	Haemolysis on BA	Lactose fermentation	Growth
MacConkey Agar							
Blood Agar							
CLED Agar							

Gram-staining test: ……

Catalase test:..…….....

Oxidase test: …….....

Coagulase test: …….....

Provisional Identification of Organism: …….

DAY 3:

Biochemical Tests Results:

Indole test	
Methyl Red test	
VogesProskauer test	
Citrate Utilisation test	
Urea Hydrolysis	
Triple Sugar Iron Agar Test	
Motility (By Hanging Drop)	
Oxidation of glucose	
Oxidation of lactose	
Oxidation of maltose	
Oxidation of mannitol	
Growth on 10% lactose agar	
Growth at 44ºC	
Lysine decarboxylase test	
Ornithine decarboxylase test	
Arginine dihydrolase	
Gelatinase liquefaction	

Further identification by Automated method (Vitek system)

Antibiotic Sensitivity Test (Kirby- Bauer Method)

Antibiotics Used	Zone Of Inhibition (mm)	Interpretation
Piperacillin(100µg)		
Ticarcillin(75µg)		
Ampicillin/Sulbactum(10/10µg)		
Piperacillin/Tazobactum(100/10µg)		
Ticarcillin-Clavulanic Acid(75/10µg)		
Ceftazidime(30µg)		
Cefepime(30µg),		
Cefotaxime(30µg)		
Ceftrixone(30µg),		
Meropenem(10µg),		
Imipenem(10µg)		
Colistin		
Gentamycin(10µg)		
Tobramicin(10µg)		
Amikacin(30µg)		
Netillimycin		
Tetracycline(30µg)		
Doxycyline(30µg)		
Ciprofloxacin(5µg)		
Levofloxacin(5µg)		
Cotrimoxyzole(1.25/23.75µg)		
Tigecycline		

Comments on Drug Resistance Pattern: ESBL/ MDR/ Non MDR

Phenotypic identification of ESBL producing isolates

- Double disk Synergy Test method (DDST)

Phenotypic identification of MBL producing isolates

- Double disk Synergy Test method
- Modified Hodge Test

Identification of MDR-*Acinetobcter baumannii*

- Culture on CHROM Agar for MDR AB

Detection for Diversity in Genes of MBL Producing Acinetobacter

Primers are

Primers	**Primers Sequence**	**bp**	**Referance**
IMP 1	F5`ACCGCAGCAGAGTCTTTGCC3` R`ACAACCAGTTTTGCCTTACC3`	448 bp	162
VIM-1	F 5`GGGAGCCGAGTGGTGAGT3` R 5`GGCACAACCACCGTATAG3`	519 bp	162
OXA 51	F 5`TAATGCTTTGATCGGCCTTG3' R 5`TGGATTGCACTTCATCTTGG3`	353 bp	162
NDM-1	F5′CTGAGCACCGCATTAGCC-3′ R5′GGCCGTATGAGTGATTGC-3′	621bp	163

Performed by

……

Checked by

……..

ANNEXURES III

CLSI-Table 2B-2 CLSI guideline for Acinetobacter spp

Table 2B-2. Zone Diameter and MIC Interpretive Standards for *Acinetobacter* spp.

Testing Conditions	Routine QC Recommendations (See Tables 3A and 4A for acceptable QC ranges.)
Medium: Disk diffusion: MHA Broth dilution: CAMHB Agar dilution: MHA	*Escherichia coli* ATCC® 25922 *Pseudomonas aeruginosa* ATCC® 27853
Inoculum: Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard	*Escherichia coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)
Incubation: 35 ± 2°C, ambient air, 20 to 24 hours, all methods	

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and 5 disks on a 100-mm plate (see M02, Section 9.2). Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
PENICILLINS									
B	Piperacillin	100 µg	≥21	18–20	≤17	≤16	32–64	≥128	
O	Mezlocillin	75 µg	≥21	18–20	≤17	≤16	32–64	≥128	
O	Ticarcillin	75 µg	≥20	15–19	≤14	≤16	32–64	≥128	
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS									
A	Ampicillin-sulbactam	10/10 µg	≥15	12–14	≤11	≤8/4	16/8	≥32/16	
B	Piperacillin-tazobactam	100/10 µg	≥21	18–20	≤17	≤16/4	32/4–64/4	≥128/4	
B	Ticarcillin-clavulanic acid	75/10 µg	≥20	15–19	≤14	≤16/2	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) [including cephalosporins I, II, III, and IV. Please refer to Glossary I.]									
A	Ceftazidime	30 µg	≥18	15–17	≤14	≤8	16	≥32	
B	Cefepime	30 µg	≥18	15–17	≤14	≤8	16	≥32	
B	Ceftaxime	30 µg	≥23	15–22	≤14	≤8	16–32	≥64	
B	Ceftriaxone	30 µg	≥21	14–20	≤13	≤8	16–32	≥64	

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Table 2B-2. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	I	R	MIC Interpretive Criteria (µg/mL) S	I	R	Comments
CARBAPENEMS									
A	Imipenem	10 µg	≥16	14–15	≤13	≤4	8	≥16	
A	Meropenem	10 µg	≥16	14–15	≤13	≤4	8	≥16	
LIPOPEPTIDES									
O	Polymyxin B	–	–	–	–	≤2	–	≥4	
O	Colistin		–	–	–	≤2	–	≥4	
AMINOGLYCOSIDES									
A	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
A	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
B	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Netilmicin	–	–	–	–	≤8	16	≥32	
TETRACYCLINES									
(2) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
B	Tetracycline	30 µg	≥15	12–14	≤11	≤4	8	≥16	
B	Doxycycline	30 µg	≥13	10–12	≤9	≤4	8	≥16	
B	Minocycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	
FLUOROQUINOLONES									
A	Ciprofloxacin	5 µg	≥21	16–20	≤15	≤1	2	≥4	
A	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥18	15–17	≤14	≤2	4	≥8	
FOLATE PATHWAY INHIBITORS									
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

DEPARTMENT OF PHARMACOLOGY
RAMA MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE
MANDHANA, KANPUR.

Date:14/03/16

TO WHOMSOEVER IT MAY CONCERN

As per the Medical Ethics Committee meeting conducted on 12/03/16, at Rama Medical College Kanpur, ethical clearance is given to the **Ph. D Student – Ms. Nidhi Pal**, from the **department of Microbiology**, for conducting her research work, titled:

"Clinical Impact of Nosocomial Infection Due to Acinetobacter baumannii in Intensive Care Units with its Antimicrobial Resistance and Molecular Characterisation."

Dr. Vinod Kumar
(Professor Anatomy)
Chairperson MEC
RMCH&RC
Kanpur, U.P